Application of Minimal Sequence Quality Values Prevents Misidentification of the *bla*_{SHV} Type in Single Bacterial Isolates Carrying Different SHV Extended-Spectrum β -Lactamase Genes

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Nucleotide sequencing is the standard molecular method for determination of the β -lactamase gene present in an isolate. Using minimal sequence quality values prevents misidentification of *bla*_{SHV} genes, as illustrated by three strains of three different species that each contained two different *bla*_{SHV} alleles, SHV-2 and SHV-12.

SHV β -lactamases, encoded by *bla*_{SHV} genes, confer resistance to a broad spectrum of β -lactam antimicrobial agents and are of significant therapeutic concern for infections caused by many species of gram-negative bacteria (4, 9, 11). At present, there are more than 50 varieties of SHV β -lactamases (7), the majority of which confer the extended-spectrum β -lactamase (ESBL) phenotype (10, 13, 14).

Several molecular methods for the detection and differentiation of different SHV β -lactamases have been proposed, but nucleotide sequencing remains the standard for determination of the β -lactamase gene present in a strain (4). Although automated thermal cycling has improved sequence analysis of *bla*_{SHV} genes (3), the quality of nucleotide sequencing data may vary, e.g., due to template quality. Usually, overall low-quality sequence data are easily identified and not used for further analysis, as are the low-quality starts and ends of sequence reads. However, quality values (e.g., Phred values [5]) are also assigned for individual positions by most sequence analysis software, but these are seldom reported. Minimal standards corresponding to a Phred value of 40 have been proposed for (human) whole-genome sequencing and for human clinical samples, but they are not uniformly applied to sequencing of bacterial targets. In this report we describe how careful interpretation and assessment of quality values prevented the misidentification of two different *bla*_{SHV} alleles in single bacterial isolates.

During a study on ESBL epidemiology (1), PCR products for β -lactamase genes of three ESBL-positive clinical strains of three different species, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*, were amplified and sequenced with the Big Dye sequencing kit 1.1 (Applied Biosystems, Foster City, Calif.) as described previously (1). The sequence assembly of the individual sequence traces for each sample, generated with Codoncode Aligner 1.3.4 (CodonCode Corporation, Dedham, MA) us-

ing default parameters, yielded an SHV-2 gene for the *E. coli* and *E. cloacae* isolates and an SHV-5 gene for the *P. aeruginosa* isolate. Sequence assemblies of sequence traces of PCR products often show low-quality positions near the ends of the assembly. This was also the case in the present analyses. However, the present assemblies contained three additional low-quality positions (Fig. 1). Visual inspection of the individual trace files showed two overlapping signals at these three positions: A or T at position 92, A or G at position 402, and A or G at position 703 (Fig. 2A). The polymorphisms at positions 92 and 703 lead to amino acid polymorphisms at positions 35 and 240 in the numbering scheme of Ambler and colleagues (2). The four possible different combinations correspond to SHV types SHV-2 (L₃₅, E₂₄₀), SHV-2a (Q₃₅, E₂₄₀), SHV-5 (L₃₅, K₂₄₀), and SHV-12 (Q₃₅, K₂₄₀) (<http://www.lahey.org/Studies/>), which differ in spectrum and activity. A possible explanation for the double signals could be that two or more *bla*_{SHV} alleles were amplified from each isolate, resulting in a mixture of templates in the sequence reaction mixture.

To assess the number and type of *bla*_{SHV} alleles in the PCR

FIG. 1. Graphic representation of Phred quality values for the contig assembled from four separate trace files for one of the *bla*_{SHV} PCR products. The quality value scale is logarithmic: a Phred value of 20 means a 1/100 likelihood of error, a Phred value of 40 means 1/10,000, and so on. The low-quality values for nucleotide positions 92, 402, and 703 are indicated.

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FIG. 2. Low-quality positions in sequence traces of a *bla*_{SHV} PCR product. (A to C) Electropherograms of the regions containing nucleotide positions 92, 402, and 703. (A) Double signals in the PCR amplicon. (B) Signals in one class of clones, T₉₂A₄₀₂G₇₀₃, corresponding to SHV-12. (C) Signals in the other class of clones, A₉₂G₄₀₂A₇₀₃, corresponding to SHV-2.

amplicons, the alleles were ligated into cloning vector pCR 2.1 (Invitrogen, Breda, The Netherlands). Ligation products were transformed to *E. coli* DH5 α . Insert size was confirmed by colony PCR with the vector primers M13F (5' GTA AAA CGA CGG CCA G 3') and M13R (5' CAG GAA ACA GCT ATG AC 3'). Each clone is the result of one individual DNA molecule from the population of *bla*_{SHV} alleles in the PCR amplicon. Eight different clones for each of the PCR amplicons of the three different isolates were sequenced, and the signals at nucleotide positions 92, 402, and 703 were compared. Only two combinations of the three polymorphisms were detected in the 24 sequenced clones (Fig. 2B and C). A₉₂G₄₀₂A₇₀₃, corresponding to SHV-2, was detected in four clones derived from the *P. aeruginosa* amplicon, five clones derived from the *E. coli* amplicon, and four clones from the *E. cloacae* amplicon. The remaining clones all contained the combination T₉₂A₄₀₂G₇₀₃, corresponding to SHV-12. The distribution among the clones suggests that these two SHV genes were present in approximately identical copy numbers in each of the three isolates.

The initial analysis of the sequence traces, based on the sequence assembly using default parameters, identified an SHV-2 gene in the *E. coli* and *E. cloacae* strains and an SHV-5 gene in the *P. aeruginosa* strain. This indicates that the use of default sequence assembly parameters without careful inspection of individual low-quality positions may lead to misidentification of the number and type of SHV genes carried by a single bacterial isolate.

To our knowledge, this is the first report of two *bla*_{SHV} genes in a single *P. aeruginosa* isolate. Only recently, the presence in a single *E. cloacae* isolate of two different *bla*_{SHV} genes that differ by a single nucleotide, SHV-7 and SHV-30, was reported (12). Szabo and colleagues developed a method to detect this single-nucleotide polymorphism by fluorescence resonance energy transfer real-time PCR. Unfortunately, adapting this method to detect the SHV-2 and SHV-12 combination in the present isolates will be impossible, as these alleles contain three nucleotide polymorphisms, which theoretically give rise to four different combinations.

The presence of the two β -lactamases in one strain has implications for its phenotype. The lysine residue at position 240 in SHV-12 is critical for the efficient hydrolysis of cefotaxime (6). In addition, the hydrolysis rate of aztreonam by SHV-12 is superior to that of SHV-2 (8). Therefore, proper identification of the SHV ESBL genes is important for optimal assessment of the cephalosporin sensitivity of a given strain. In addition, full understanding of the epidemiology of extended-spectrum β -lactamases requires accurate data on the presence of the different types of these enzymes in various species. Hence, careful evaluation of low-quality values (Fig. 1 and 2) in individual sequence traces is warranted. This is particularly relevant for positions where polymorphisms are known to cause changes in the spectrum of SHV β -lactamases, i.e., the positions corresponding to amino acid positions 35, 238, and 240 (4). Identification of multiple alleles can be crucial to identify outbreaks of resistance plasmids that carry ESBL genes and is essential to understand the dynamics and the evolution of these genes.

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